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Neurons and glia arise from a common progenitor in chicken optic tectum: Demonstration with two retroviruses and cell type-specific antibodies

(β-galactosidase/nuclear signal/neurogenesis/gliogenesis)

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ABSTRACT **We used a recombinant retrovirus to study cell lineage in the chicken optic tectum. The virus inserts the *Escherichia coli lacZ* (β-galactosidase) gene into the genome of an infected cell; a histochemical stain marks the progeny of infected cells with a blue precipitate. We had previously shown that individual clones frequently contain diverse neuronal types. Now we asked whether individual clones contain glia as well as neurons. To this end, we constructed a virus in which *lacZ* is fused to a nuclear localization signal sequence from the simian virus 40 large tumor antigen. Cells infected with this virus are marked with blue nuclei instead of blue somata. In embryos injected with a mixture of the two retroviruses, individual clusters contained cells with only one label type (nuclear or cytoplasmic), thus verifying that clusters of cells were clones. Furthermore, it was possible to immunostain the somata of cells that had blue nuclei, whereas the blue cytoplasmic precipitate hampered immunostaining. Together, these methods allowed us to show that some clones contained neurons (neurofilament-positive) and two types of glia (glutamine synthetase-positive and glial fibrillary acidic protein-positive). This result demonstrates the existence of a common progenitor for neurons and glia in optic tectum.**

A few years ago, we (1) and Price *et al.* (2) devised a method of cell lineage analysis in which a recombinant retrovirus is used to insert the *Escherichia coli lacZ* gene into the genome of a dividing cell; the product of the gene, a β-galactosidase (LacZ), is later detected histochemically in the progeny of the infected cell (for review, see ref. 3). In one application of this technique, we injected retrovirus into the developing brain of chicken embryos and subsequently analyzed the arrangement and phenotypes of clonally related cells in the optic tectum. Individual clones of $LacZ^+$ cells frequently spanned many laminae and contained neurons of diverse morphologies, showing that at least some tectal progenitors can generate several neuronal types (4).

A logical next step in this analysis is to ask whether individual progenitors can give rise to glia as well as to neurons. However, our attempts to answer this question were thwarted by two limitations of the technique. (*i*) It was difficult to identify $LacZ^+$ cells as glia. Although some $LacZ^+$ cells were readily recognizable as neurons by their size and shape (4), glia were difficult to distinguish from small neurons by these criteria. We expected cell type-specific antibodies to help in this regard, but immunohistochemical signals from fluorochrome- and enzyme-conjugated antisera were obscured by the dense blue reaction product of the histochemical stain for LacZ. (*ii*) It was sometimes difficult to ascertain clonal boundaries. Although clonally related neurons usually form coherent radial arrays (4), many putative glia were displaced tangentially. It was therefore unclear whether the glia had migrated tangentially from their neuronal relatives or arisen from separate, nearby progenitors. Thus, our tentative conclusion, that some tectal progenitors give rise to both neurons and glia, remained unverified because of ambiguities in the identification of glial cells and of clonal boundaries.

To circumvent both of these problems, we constructed a retroviral vector that targets LacZ to the nucleus. This was done by replacing the *lacZ* gene in the virus with a construct in which *lacZ* is fused to a short "signal" sequence from the nuclear large tumor antigen of the simian virus 40 virus (5–7); in fact, Kalderon *et al.* (5) originally used this fusion to show that the sequence in question codes for a nuclear localization signal (5). As expected, cells infected with the nuclear signal-*lacZ* (*nslacZ*) virus had blue nuclei instead of blue somata after staining. We were then able to use the *nslacZ* virus in two types of double-staining protocols. (*i*) Embryos were infected with a mixture of *lacZ* and *nslacZ* viruses to determine whether individual clusters of cells presumed to represent clones indeed contained cells with only one type of label, nuclear or cytoplasmic. (*ii*) We combined immunofluorescent visualization of cytoplasmic antigens with β-galactosidase histochemistry to determine the phenotype of cells that had blue nuclei. Together, these methods allowed us to demonstrate unambiguously that some clones of tectal cells contain both neurons and glia.

METHODS

Viruses. The recombinant retroviral vectors used in this study (Fig. 1) were constructed as follows. pLZ10: A *lacZ* gene (8) was modified to insert a *Bam*HI site at amino acid 8. The *Bam*HI–*Dra* I fragment of *lacZ* was then inserted between the *Bgl* II and *Pvu* II sites of a rearranged form of the SRA-2 clone of the Schmidt–Ruppin A strain of Rous sarcoma virus (RSV) (9). This fuses *lacZ*, in frame, to a large fragment of the RSV *gag* gene. pLZ11: Because sequences in the *gag* gene are required for proper assembly of the retrovirion (10), and because we did not know whether the nuclear signal (ns) would function at an internal position (i.e., gag-ns-LacZ; see ref. 11), we constructed a vector in which *nslacZ* was placed downstream of *gag*, under the control of an internal RSV promoter. *Bgl* II linkers were added to L7RH-βGal (5) at the *Sfi* I site located towards the 3′ end of the simian virus 40 promoter. Subsequent cleavage with *Sac* I yielded a fragment containing the large tumor antigen-derived nuclear localization sequence and approximately two-thirds of the *lacZ* gene. This *Bgl*

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Abbreviations: RSV, Rous sarcoma virus; LacZ, *Escherichia coli* β-galactosidase; nsLacZ, *E. coli* β-galactosidase fused to nuclear localizing sequence from the simian virus 40 large tumor antigen; E (with number), embryonic day; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactosidase.

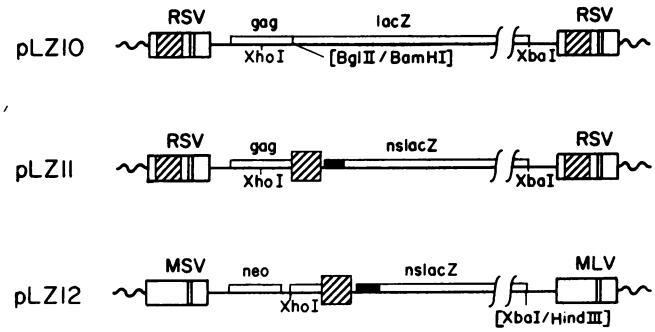

FIG. 1. Retroviral vectors used in this study. Virus LZ10, encoded by pLZ10 produces cytoplasmic, soluble LacZ, whereas the viruses LZ11 and LZ12 produce a nsLacZ fusion protein that is directed to the nucleus. RSV, Moloney sarcoma virus (MSV), and Moloney leukemia virus (MLV) long terminal repeats are boxed. Black boxes indicate nuclear signal (ns). Wavy lines indicate bacterial plasmid sequences. The diagonal lines mark the RSV sequences used as internal promoter. Key restriction sites used in the constructions are marked; brackets indicate sites destroyed by ligation. Diagrams are not to scale.

II–*Sac* I fragment was ligated to a *Sac* I–*Hin*dIII fragment from pLZ10 containing the remainder of the *lacZ* gene and 3′ RSV long terminal repeat plus *E. coli* plasmid sequences, and to a *Hin*dIII–*Bgl* II fragment containing the 5′ RSV long terminal repeat *gag* sequences, and a second, truncated RSV long terminal repeat extending from −10 to −200 that includes characterized enhancer and promoter elements but lacks integration sequences and the polyadenylylation signal. pLZ12: A *Xho* I–*Xba* I fragment from pLZ11 containing the truncated RSV promoter and sequences encoding the nuclear-targeted LacZ was inserted between the *Xho* I and *Hin*dIII sites of the Moloney murine retroviral vector, pLNL6 (12), after partially filling in the *Xba* I and *Hin*dIII sites to make them compatible.

Viruses LZ10 and LZ11 were produced by cotransfecting the appropriate plasmid, a gene (*neo*) that confers resistance to the antibiotic G418, and helper plasmid into QT6 quail fibroblasts. The helper plasmid was derived from the SRA-2 clone of RSV (9): bases 45–260 (numbered as in the PrC strain in ref. 13) were deleted to remove the packaging signal; most of the *src* gene and the entire 3′ long terminal repeat were removed by cutting at the *Pvu* II site at base 7803 (13), and a polyadenylylation signal was provided by adding the *Hinc*II–*Bam* fragment from the simian virus 40 (14). LZ12, which contains both *neo* and *lacZ* genes, was produced in the amphotropic helper cell line PA317 (15), obtained from American Type Culture Collection. Virus-producing cells were selected with G418, cloned, tested, and maintained as described in ref. 1. Virus collected from culture medium was concentrated by centrifugation for 2 (LZ10) or 4 (LZ12) hr at 15,000 rpm in a Beckman SW28 rotor; the pellet was resuspended in ≈1% of its original volume of culture medium, divided into aliquots, and stored frozen in liquid N_2. The titers of the concentrates were $\approx 10^6$ active virions per ml, as determined by infection of cultured fibroblasts (1). Viral stocks were tested for the presence of replication-competent recombinants by transferring the supernatants of virus-infected cultures to other fibroblasts and subsequently staining the latter for LacZ; no replication-competent virus was ever detected.

Surgery. Fertilized White Leghorn chicken eggs from SPAFAS (Roanoke, IL) were incubated at 37°C and staged by the system of Hamburger and Hamilton (16). Viral concentrates were injected into embryos as described in ref. 4.

Histology. For experiments on skin, whole embryos were fixed in 2% (wt/vol) paraformaldehyde and 0.4% glutaraldehyde in phosphate-buffered saline (150 mM sodium chloride/15 mM sodium phosphate, pH 7.3) for 1 hr. The embryos were then stained for LacZ at room temperature overnight in the dark in a solution containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) at 1 mg/ml, 20 mM potassium ferrocyanide, 20 mM potassium ferricyanide, and 2 mM $MgCl_2$ in phosphate-buffered saline. Subsequently, skin areas bearing $LacZ^+$ cells were identified under the dissecting microscope and mounted in glycerol. For experiments on tectum, brains were sectioned on a Vibratome (Ted Pella, Inc., Redding, CA) after fixation and before staining in X-Gal. When tecta were to be immunostained, the fixative contained 0.01% instead of 0.4% glutaraldehyde. Then, after staining with X-Gal, Vibratome sections bearing $LacZ^+$ cells were infiltrated with 30% (wt/vol) sucrose, frozen in liquid N_2, and resectioned at 6 μm in a cryostat. The cryostat sections were mounted on glass slides, air-dried, and incubated successively with primary and secondary antibodies. Primary antibodies were rabbit anti-dephosphorylated chicken neurofilament M protein (17), mouse monoclonal anti-chicken carbonic anhydrase C (18), rabbit anti-bovine glial fibrillary acidic protein (Dakopatts, Denmark), and rabbit anti-chicken glutamine synthetase (19, 20). Secondary antibodies were fluorescein- and rhodamine-conjugated goat anti-mouse IgG and anti-rabbit IgG (Sigma and Boehringer Mannheim, Indianapolis, IN). Finally, the sections were mounted in glycerol/*p*-phenylenediamine.

RESULTS

LacZ Delivered to Cytoplasm or Nucleus. In previous studies of cell lineage in chicken optic tectum (4), we used a recombinant RSV, LZ10 (Fig. 1). LZ10 vector encodes a gag-LacZ fusion protein that is freely soluble in phosphate-buffered saline (data not shown) and stains the cytoplasm of cells more intensely than their nuclei (Fig. 2 *a* and *c* and Fig. 3 *a*, *c*, and *e–g*). To direct LacZ to the nucleus, we constructed a vector in which the nuclear localizing signal from the simian virus 40 large tumor antigen was fused to the N terminus of LacZ (pLZ11; see *Methods*). pLZ11-transfected helper cells produced virions, and the nucleus was far more intensely $LacZ^+$ than the cytoplasm in LZ11-infected fibroblasts. However, the LZ11 titer was low, and the number of $LacZ^+$ cells per clone was ≈50% lower for virus LZ11 than for LZ10. These results suggested either that high levels of nsLacZ were toxic or that interactions among the promoters in the vector were inactivating transcription of retroviral and *lacZ* genes (see ref. 21). We therefore constructed another vector, pLZ12, in which *nslacZ*, under the control of the RSV promoter, was placed into a Moloney murine retrovirus vector, in which interactions between promoters are apparently less severe (22). Virus LZ12 directed expression and nuclear localization of LacZ in skin (Fig. 2 *b* and *d*), optic tectum (Fig. 3 *b*, and *d*), and numerous other cell types (23–25); the titer of LZ12-producing clones remained stable for months; and there were similar numbers of $LacZ^+$ cells per clone in LZ10- and LZ12-infected fibroblasts in culture (data not shown) and in LZ10- and LZ12-infected skin cells *in vivo* (Table 1). Thus, by criteria that have been detailed (1), LZ12 appears to be a suitable virus for lineage analysis.

Paired Retroviruses Used to Ascertain Clonal Boundaries. In previous work, groups of $LacZ^+$ cells have been asserted to be clonally related when they form coherent clusters separated by arbitrarily long distances from other clusters (1–4, 26, 27). We used skin, which is easy to analyze in whole mounts of tissues, to show that retrovirus pairs can provide better evidence that clusters are clones. When chicken skin was stained for LacZ on embryonic day 8 (E8) after injection of either virus LZ10 or LZ12 at E2, a pattern similar to that reported for mouse skin (1) was seen: clusters of closely spaced, but not necessarily adjacent $LacZ^+$ cells were separated by several cluster-diameters from other labeled cells

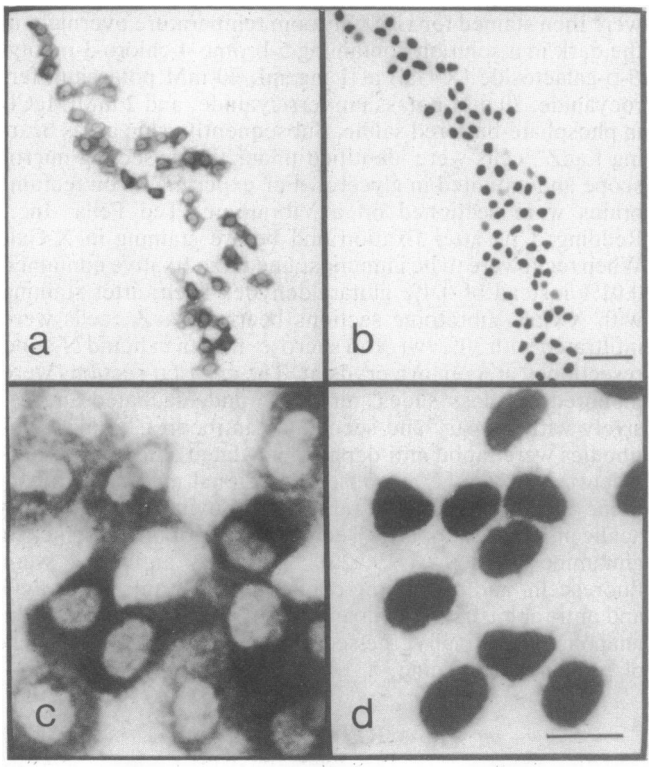

FIG. 2. LacZ$^+$ cells from the skin of chickens infected with the recombinant virus LZ10 (cytoplasmic LacZ) (*a* and *c*) or LZ12 (nuclear LacZ) (*b* and *d*). (*c* and *d*) Higher power micrographs of cells from clusters similar to those shown in *a* and *b*. Individual cells are readily classified as having stained nuclei or cytoplasm, and all cells within individual clusters are homogeneous in staining pattern. (Bar = 100 μm for *a* and *b* and 50 μm for *c* and *d*.)

(Fig. 2 *a* and *b*). We then mixed roughly equal numbers of LZ10 and LZ12 virions (based on titers obtained with cultured chicken fibroblasts), injected the mixture into embryos, and again analyzed the skin at E8. If each cluster of LacZ$^+$ cells arose from a single progenitor, it should contain cells with stained cytoplasm *or* cells with stained nuclei but not both types. If, however, more than one progenitor gave rise to a cluster, at least some clusters should contain a mixture of cytoplasm-positive and nucleus-positive cells. In fact, no such mixed clusters were found (Table 1), even though both nucleus-positive and cytoplasm-positive clusters were found in each animal. Statistical analysis of these results (see Table 1 legend) validated the use of paired retroviruses in delineating clonal boundaries and confirmed that discrete clusters of LacZ$^+$ cells represent clones.

Immunohistochemical Staining of Cells with LacZ$^+$ Nuclei. Attempts to counterstain cytoplasmic LacZ$^+$ cells immunohistochemically met with little success: in most cases, the blue X-Gal product obscured the immunohistochemical marker (data not shown). Although antibodies to LacZ can be used instead of the histochemical stain (e.g., ref. 27), they are unsuitable for identifying LacZ$^+$ cells in whole mounts or thick sections of tissues, through which the histochemical stain penetrates well but antibodies penetrate poorly. However, it proved straightforward to immunohistochemically stain the cytoplasm surrounding the blue nuclei of LZ12-infected cells (Fig. 4). Thus, cell type-specific antibodies can be used in conjunction with LacZ histochemistry to determine the phenotypes of clonally related cells.

Coexistence of Neurons and Glia in Single Tectal Clones. To learn whether individual tectal progenitors give rise to both neurons and glia, we first used mixtures of LZ10 and LZ12 viruses to ascertain clonal boundaries. Virus was injected into the tectal ventricle at E3 (stages 16–18), and tecta were fixed and stained for LacZ at E7–8 (stages 30–33) or E18–19 (stages 44–45). On E7–8, cells were arranged in strictly radial arrays; as expected from previous evidence that radial arrays are clones (4), each array contained cytoplasm-positive or

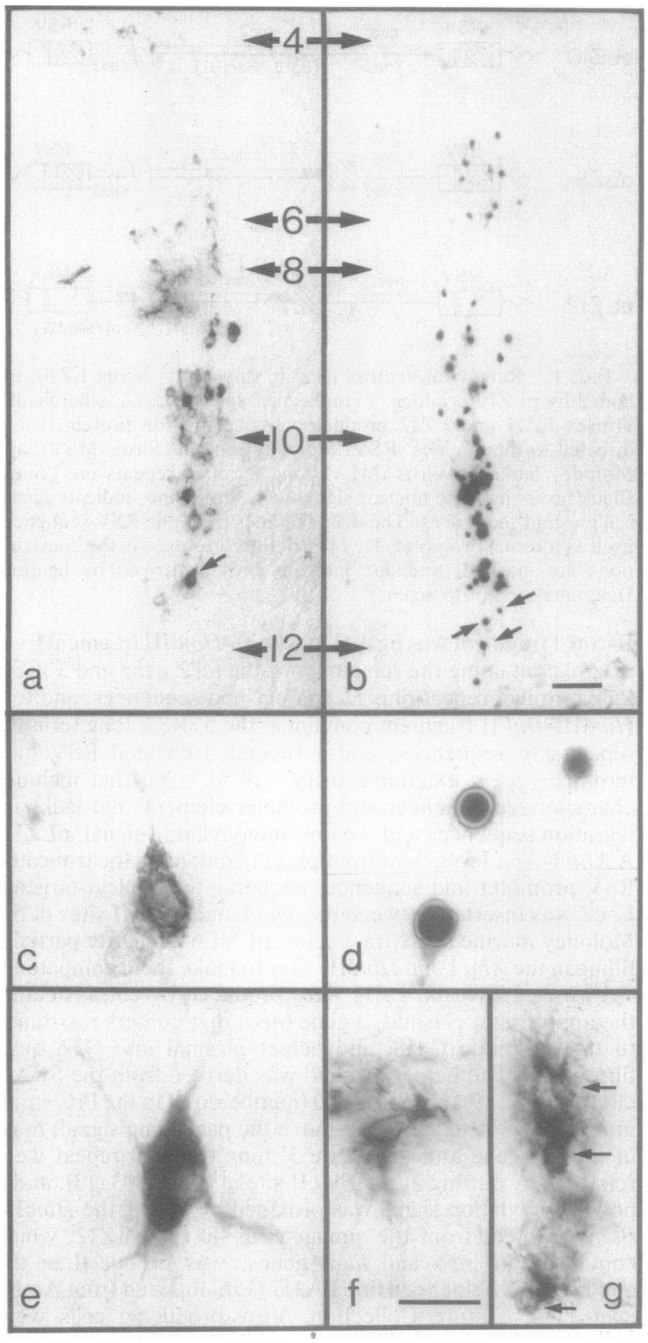

FIG. 3. LacZ$^+$ cells in optic tecta of E18 embryos injected with retrovirus on E3. (*a* and *b*) Low-power micrographs of clones. In *a*, all cells have stained cytoplasm (i.e., are derived from an LZ10-infected progenitor), whereas in *b*, all cells have stained nuclei (i.e., are derived from an LZ12-infected progenitor). Pial surface is up, ventricular surface is down, and several laminae (numbered as in ref. 4) are marked. (*c* and *d*) High-power micrographs of cells marked by arrows in *a* and *b* to show the clear distinction between nuclear and cytoplasmic staining. Note that the presence of faint cytoplasmic staining in the lowest cell in *d* does not prevent classification of this cell as nucleus-positive. (*e–g*) Cells from LZ10-marked clones, which show the morphological distinction between neurons (*e*; also *c*) and glia (*f* and *g*). Glia sometimes occur singly (*f*) but are frequently clustered (*g*); arrows indicate glial somata within the cluster. (Bar = 80 μm in *a* and *b* and 25 μm in *c–g*.)

Table 1. Paired retroviruses delineate clonal boundaries in skin

Staining pattern	Number of clusters	Cells per cluster
Nuclear	37	88 ± 10
Cytoplasmic	35	66 ± 9
Mixed	0	

Embryos were injected with vectors LZ10 and LZ12 on E2 and analyzed at E8. Cells were scored as to whether their nucleus or cytoplasm was more intensely $LacZ^+$. Dividing cells with no nuclear membrane were not classified. One interphase cell in one "nuclear" clone had intensely stained cytoplasm. Values are means ± SE. If nuclear and cytoplasmic clusters were equally frequent (which is approximately true), the probability that all clusters arise from two or more separately infected cells is $<<0.001$, by the χ^2 test, and the probability that ≥10% of the clusters arise from two or more cells is <0.05.

nucleus-positive cells but not both types (Table 2, top). At E18–19, most arrays of $LacZ^+$ cells remained radial in orientation but were broader than those seen at E8 and often included small clusters of outlying cells (Fig. 3 *a* and *b*, and G.E.G. and J.R.S., unpublished work). In some arrays, all cells had more intensely stained nuclei than cytoplasm; in other arrays, all cells had more intensely stained cytoplasm than nuclei. No arrays contained mixtures of nucleus-positive and cytoplasm-positive cells (Table 2 and Fig. 3 *a–d*). From this result, we conclude that each cluster of $LacZ^+$ tectal cells represents a clone.

With this assurance, we used two methods to ascertain whether individual clones contain both neurons and glia. (*i*) Morphological criteria were used to identify cells in a group of 396 clones from tecta infected with the recombinant virus LZ10 at stages 14–18 and stained for LacZ at E18–21. These clones contained from 2 to ≈100 cells each, with an average of ≈10 $LacZ^+$ cells per clone. Relatively large cells with prominent nuclei, abundant cytoplasm, and long, discrete stained processes were classified as neurons (Fig. 3 *c* and *e*); small cells with irregular nuclei, a thin rim of cytoplasm, and a feather-like spray of fine short processes were classified as glia (Fig. 3 *f* and *g*); in many instances, the processes of these cells were associated with blood vessels and/or the pial surface, as expected of astrocytes. In this group, 156 of the clones (39%) contained both neurons and glia, 94 (24%) contained only neurons, 53 (13%) contained only glia, and 93 (23%) contained either neurons or glia plus some cells that were not identifiable by morphological criteria alone. Thus, over one-third of the tectal progenitors marked on E3 gave rise to both neurons and glia.

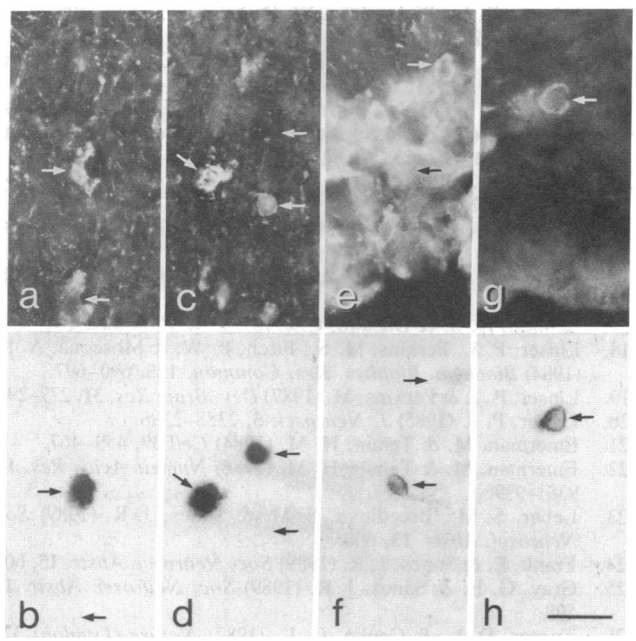

FIG. 4. Immunological identification of LZ12-stained cells as neurons or glia. (*a–f*) Three near-serial sections from a single clone at the same magnification. One section (*a* and *b*) was stained with anti-neurofilaments; the second section (*c* and *d*) was stained with anti-glutamine synthetase; and the third was stained with anti-glial fibrillary acidic protein (*e* and *f*). (*g* and *h*) Carbonic anhydrase-positive glial cell from another clone. Each section was photographed with bright-field (*a*, *c*, *e*, and *g*) and fluorescence (*b*, *d*, *f*, and *h*) optics. Arrows mark corresponding points on each pair of micrographs. (Bar = 25 μm.)

Table 2. Paired retroviruses delineate clonal boundaries in optic tectum

Age	Staining pattern	Number of clusters	Cells per cluster
E8	Nuclear	20	31 ± 4
	Cytoplasmic	30	27 ± 2
	Mixed	0	
E18	Nuclear	27	38 ± 5
	Cytoplasmic	12	24 ± 7
	Mixed	0	

Tectal vesicles were injected with vectors LZ10 and LZ12 on E2, and tecta were analyzed at E8 or E18. Cells were scored, and significance was assessed as in Table 1. The probability that all clusters arise from two or more separately infected cells is $<<0.001$ for each age, and the probability that ≥10% of the clusters arise from two or more cells is <0.05 for the pooled data.

(*ii*) Immunostaining was used to confirm the presence of neurons and glia in a group of clones from tecta infected with the recombinant virus LZ12. We picked nine large clones (>10 cells) suspected to contain both cell types, resectioned them, and stained sections with antibodies to neurofilaments (which are neuron-specific in tectum; Fig. 4 *a* and *b*), to glutamine synthetase (which is glia-specific in tectum; Fig. 4 *c* and *d*), to glial fibrillary acidic protein (which stains a subset of tectal astrocytes; Fig. 4 *e* and *f*), or to carbonic anhydrase C (which stains a largely nonoverlapping set of tectal glia; Fig. 4 *g* and *h*). All nine clones analyzed in this way contained both neurons and glia. In five of the clones we identified two distinct glial types: glutamine synthetase-positive astrocytes in middle laminae, and glial fibrillary acidic protein-positive astrocytes near the ventricular or pial surfaces (see refs. 19 and 20 for descriptions of these two cell types). Thus, immunostaining confirmed the multipotential nature of tectal progenitors and further revealed that individual progenitors marked on E3 can give rise to two distinct types of glia as well as to neurons.

DISCUSSION

A Common Progenitor for Neurons and Glia. We previously used retrovirus-mediated gene transfer to show that individual progenitors can give rise to multiple neuronal types in the chicken optic tectum (4). Here, we have shown that some progenitors generate glia as well as neurons. Some clones contain, in addition to neurons, two types of glia that are distinguishable immunohistochemically and by position and which may correspond to fibrous and protoplasmic astrocytes (20). We also know that some clones containing both types of glia contain three or four morphologically distinct types of neurons as well (G.E.G. and J.R.S., unpublished work). Further analysis will be necessary to learn whether particular types of neurons and glia are more or less likely to appear in combination (i.e., whether separate populations of precursors are distinguishable) and how other glial types (e.g., radial glia, microglia, and oligodendrocytes) are related to those we have detected. However, it is already apparent

that some tectal progenitors can generate projection neurons, interneurons, and at least two types of glia.

An important reason to learn when neuronal and glial lineages diverge is to deduce when and where cells become predisposed to particular fates. Accumulating evidence suggests that different parts of the central nervous system may employ different strategies in this regard. In the mammalian cerebral cortex, a distinct subset of mitotically active cells in the ventricular zone expresses a glial marker (28), and individual clones frequently contain neurons or glia but not both types (27, 29). Evidently, some degree of phenotypic commitment is induced in proliferating stem cells in this case and inherited by their progeny. In retina, on the other hand, even the terminal mitosis sometimes gives rise to a neuron and a glial cell, suggesting that phenotype is determined at or after the final division (26, 30, 31). Our results suggest that in tectum, developmental decisions resulting in the choice of a neuronal or glial fate are made after the ventricular zone has formed. The methods we have used can now be extended to learn how long progenitors remain multipotential. By analyzing clones marked by retroviral injection at successively later stages, we can ask whether neuronal and glial lineages eventually diverge (as in cortex) or whether cells remain multipotential until mitosis is over (as in retina). Combined with experiments in which tectal cell phenotypes are altered by manipulation *in vitro*, these results will provide insights into the environmental factors that influence phenotypic choices.

Improvements in the Retroviral Method of Lineage Analysis. The use of the *nslacZ* vector has proven useful in two ways. First, simultaneous application of virions encoding LacZ and nsLacZ provides a new criterion for assessing clonal boundaries. It is a fundamental limitation of the retroviral method that one has no way of proving that any given group of $LacZ^+$ cells represents the progeny of a single infected cell. In general, we have used the arrangement of $LacZ^+$ cells in widely spaced, coherent clusters to argue that each cluster is a clone (1). In three instances, statistical analyses supported this conclusion: when fewer virions were applied to a tissue, fewer clusters resulted, but the mean number of cells per cluster was unaffected (4, 26, 27). With paired viruses, we have been able to ask whether individual clusters are uniformly homogeneous with regard to viral phenotypes. Although this test cannot be used to prove definitively that any individual cluster is a clone, it does permit assessment of clonal boundaries within single animals and without reaching limiting dilution. It therefore permits the retroviral method to be applied to cases in which cells disperse as or after they divide. For example, use of this method has recently permitted us to initiate analyses of lineage in spinal cord (23) and in neural crest-derived structures (24).

In principle, the use of paired *lacZ* and *nslacZ* vectors is equivalent to the use of two distinct reporter genes—e.g., enzymes that would produce blue and red reaction products. The advantage of our method is that a single histochemical stain reveals both viral phenotypes, thus avoiding the use of separate reactions that might be incompatible, cross-reactive, or unequally sensitive. A potential disadvantage is that the nuclear and cytoplasmic staining patterns might not always be distinguishable. Unsurprisingly, cytoplasmic staining was seen in nsLacZ-infected skin cells when the cells were in metaphase and had no nuclear membranes. In addition, we observed nsLacZ encoding virus-infected cells with stained cytoplasm in $\approx$1% of presumably postmitotic tectal cells in E18–19 embryos. This might reflect slow leakage of nsLacZ from the nucleus, partial proteolysis of nsLacZ to produce LacZ, overloading of nuclear storage sites, or down-regulation of the nuclear transport system by postmitotic cells (32). Nonetheless, the nsLacZ-stained cells had more intensely stained nuclei than cytoplasm in virtually all cases, whereas LacZ-stained cells virtually always had more intensely stained cytoplasm than nuclei. Thus, at least in skin and tectum, the two viral phenotypes can be distinguished with confidence.

The second advantage of the nsLacZ label is that it facilitates immunohistochemical typing of clonally related cells. Whereas some $LacZ^+$ cell types are identifiable by virtue of their size, shape, and position (1, 4, 26, 27, 29, 33), cell type-specific antibodies are needed to positively identify others, such as the tectal glia studied here. Use of immunohistochemical stains will also be necessary to establish the clonal relationships among cellular subtypes that are morphologically indistinguishable but chemically distinct—e.g., neurons that use different transmitters.

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